THE PRODUCTION OF POLYHYDROXYALKANOATES USING THE WASTE STREAM OF AN ANAEROBIC DIGESTER

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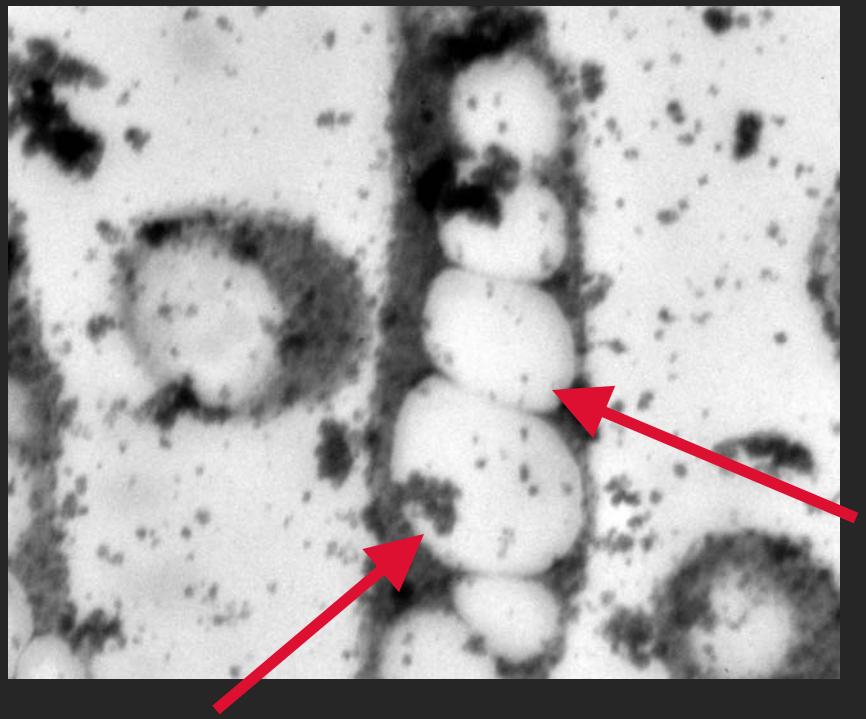
Biological and Irrigation Engineering

Introduction

- Conventional Plastics: **35-55%** of municipal solid waste sent to landfills[8]
- Rising Cost of Oil: Record highs - $**105**/barrel
- Rising Cost of Petrochemical Plastics: $1.42 to $1.75/kg

Introduction



- Polyhydroxyalkanoates (PHAs) form a class of bioplastics
- Polyhydroxybutyrate (PHB) is most prevalent

More on PHAs

- Cost: Reported values range from $2.89/kg to $16/kg
- Production cost typically decreased through the use of waste streams as carbon and nutrient source
- Carbon = 80% of raw material cost
- Carbon Waste = FREE

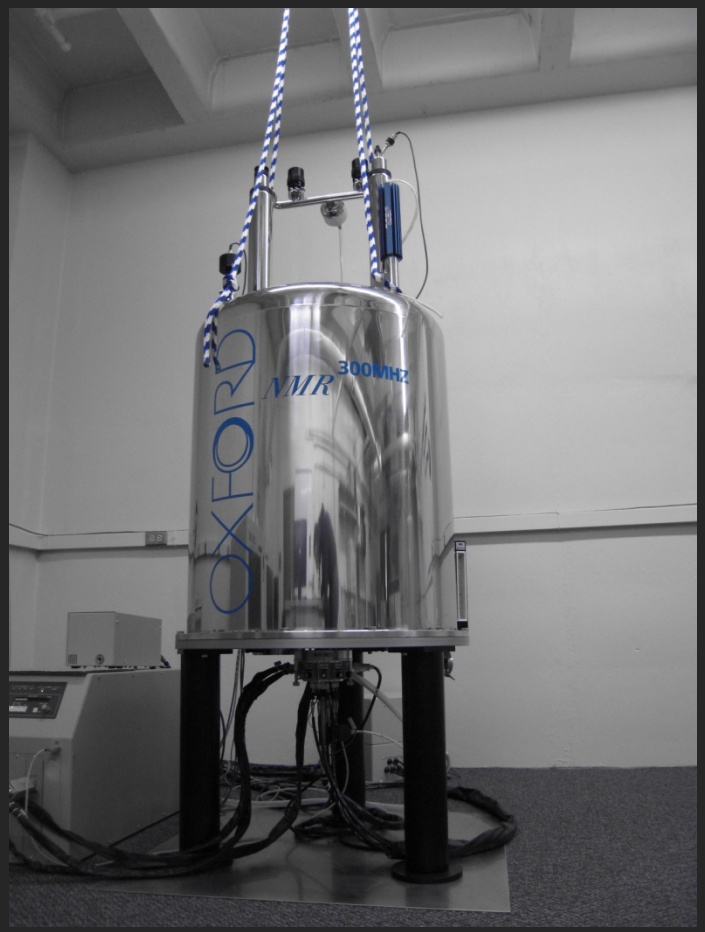
Anaerobic Digestion

- Agricultural waste treatment
- Phosphorus-accumulating organisms (PAOs)
- Need analytical methods for PHA detection

Analytical Methods

- Gas Chromatography
- High Performance Liquid Chromatography
- Fluorescence
- Nuclear Magnetic Resonance

Nuclear Magnetic Resonance



- Identify the carbon-hydrogen framework of an organic compound
- 300 MHz ^{1}H-NMR

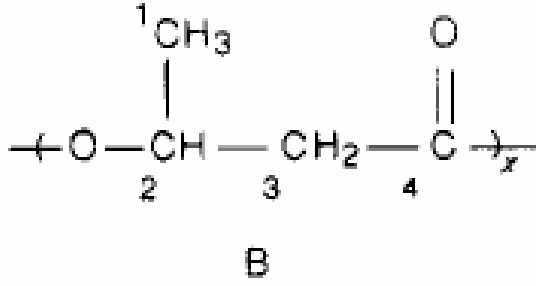
Benefits of NMR

- Rapid analysis – 5 min.
- No preliminary extraction from samples
- Non-destructive
- Independence from analysis history
- Discrimination between types of PHAs

Development and Validation of NMR for PHA

1. Polyhydroxybutyrate Standards
2. Pure Cultures (*A. vinelandii*)
3. Anaerobic Digester Samples

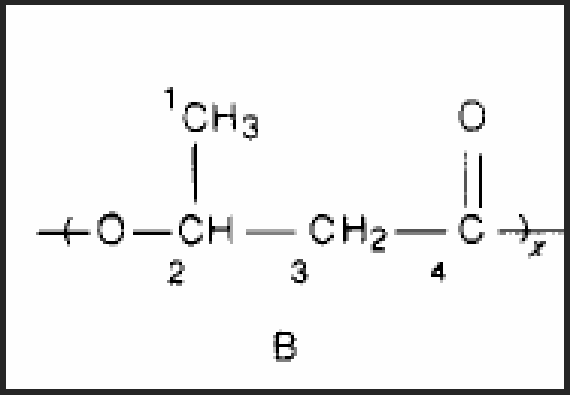
1. POLYHYDROXYBUTYRATE STANDARD

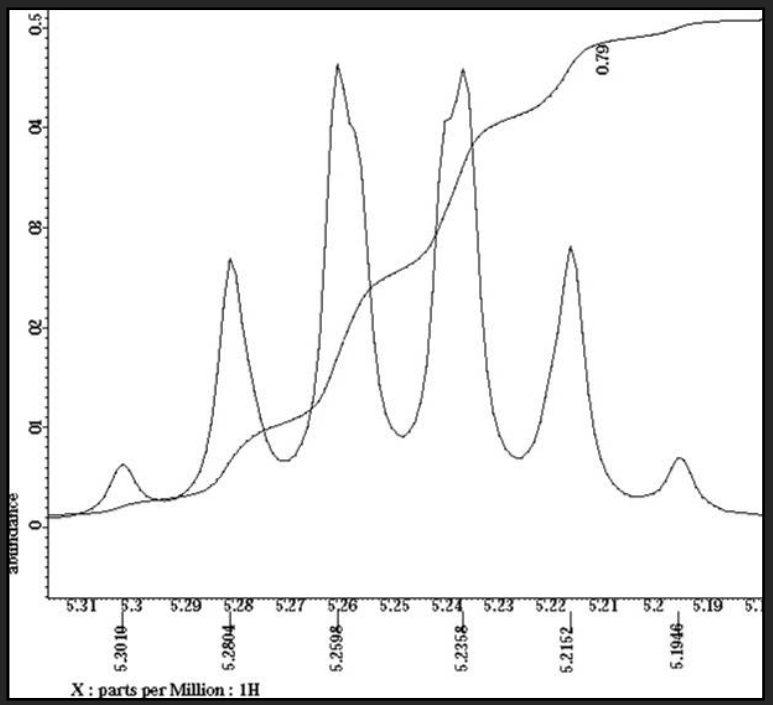


B

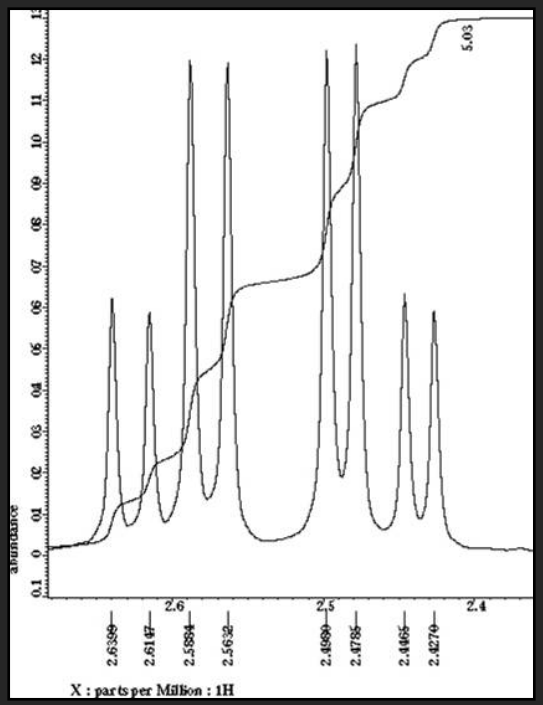
PHB Standard

- NMR Peaks Observed

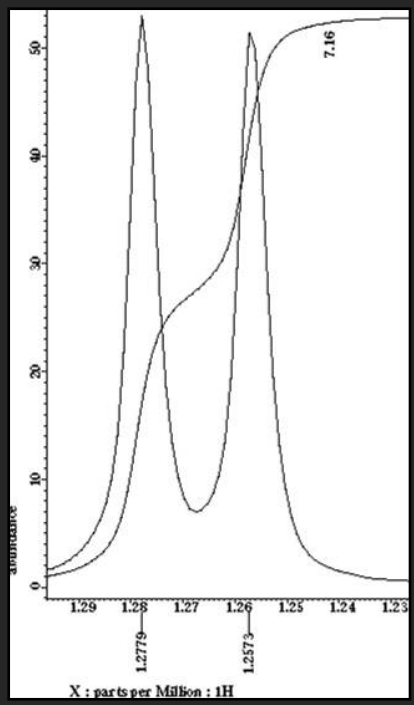




CH (2)

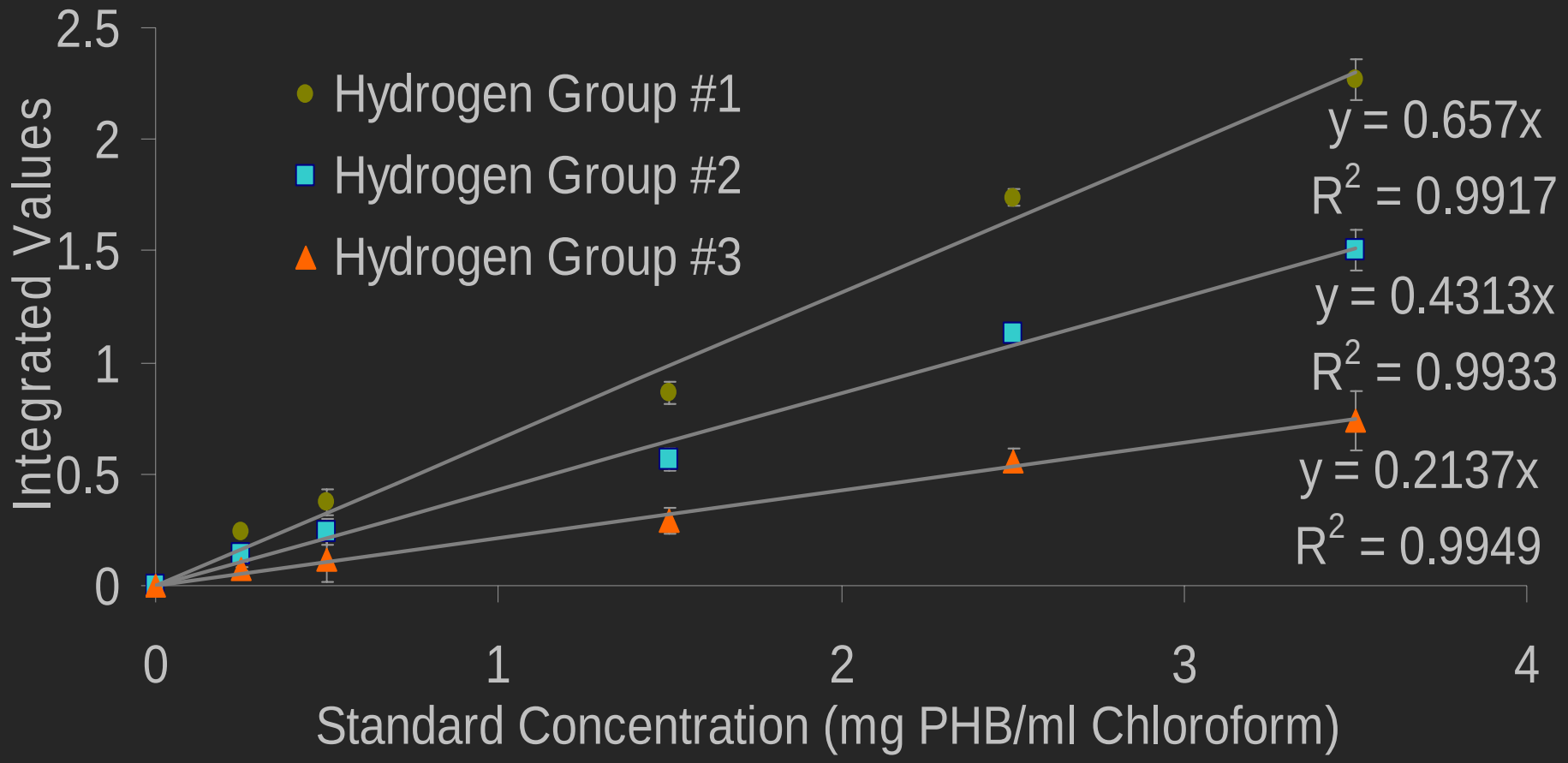


CH_2 (3)



CH_3 (1)

NMR Calibration Curve



2. Pure Culture

- *Azotobacter vinelandii* accumulates PHB aerobically during growth

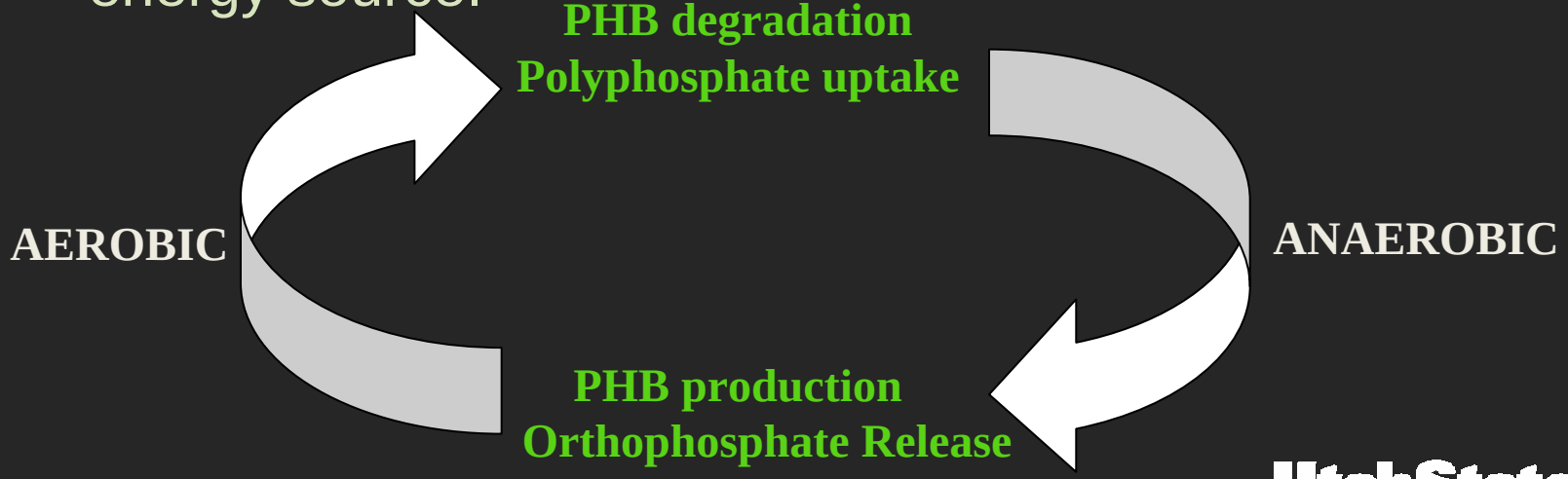
NMR with *A. vinelandii*

- Product Yield – 3.74 mg PHB/10 mg lyophilized cells

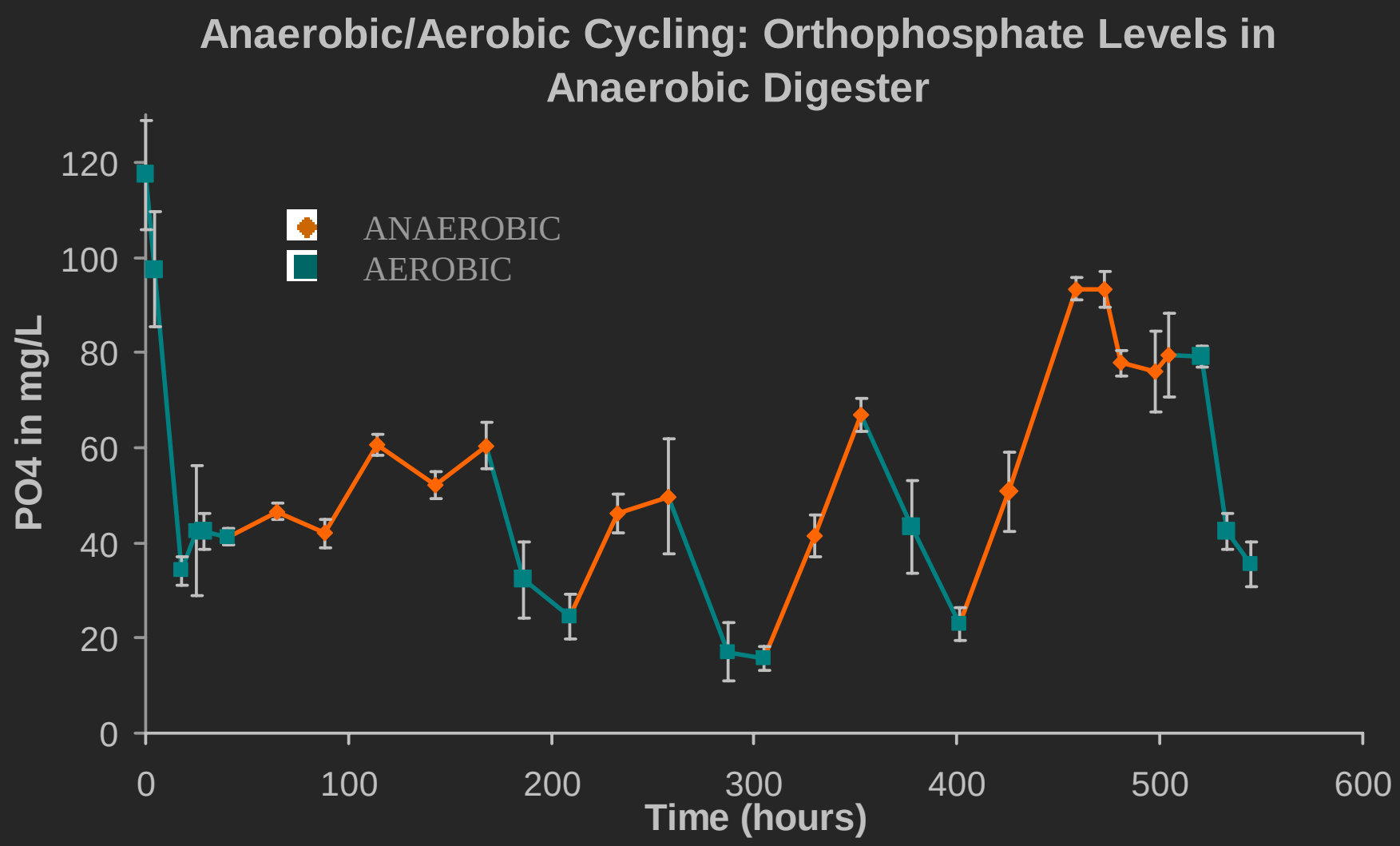
  = 37.4% PHB

3. PHA Detection in Digester

- Phosphorus Accumulating Organisms (PAOs)
  - Anaerobically, accumulate PHAs and release hydrolyzed polyphosphate in the form of orthophosphate.
  - Aerobically, PAOs take up orthophosphate to recover poly-P and utilize the stored PHAs as a carbon and energy source.



Enriched PAO Culture



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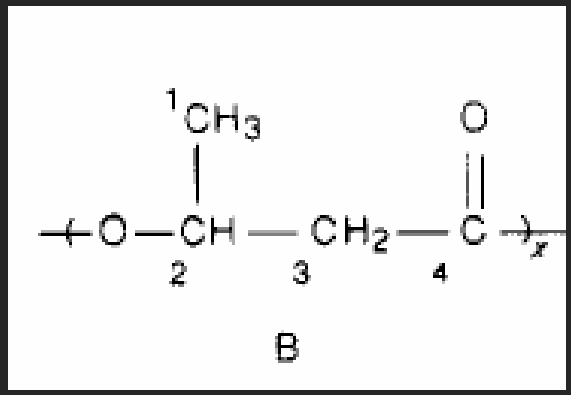
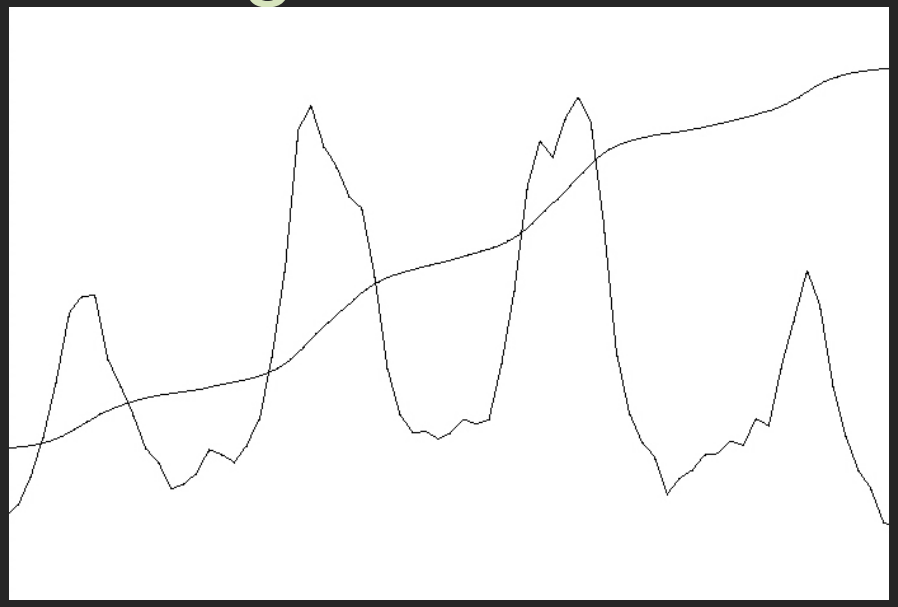
Digester NMR Results

- PHB Peaks Observed
- Yield: 0.18 mg PHB/ml of digester effluent





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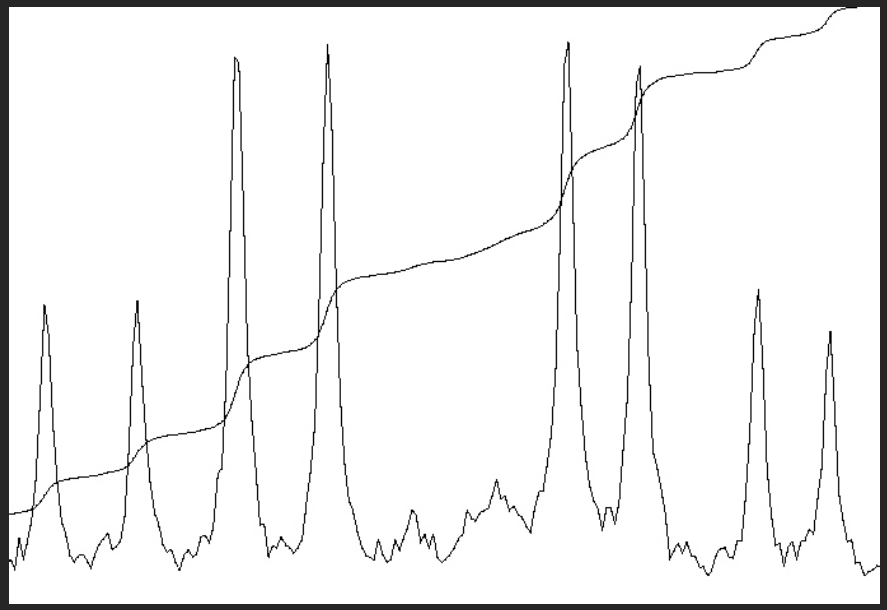

CH_2 (3)

Conclusions

- Nuclear Magnetic Resonance is a rapid and successful method for detection and quantification of PHAs.
- PHA producers are naturally present in anaerobic digester waste

Acknowledgements

- Utah Science Technology and Research initiative (USTAR)
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- Dr. Piotr Dobrowolski, USU Dept. of Chemistry
- Associated Students of Utah State University

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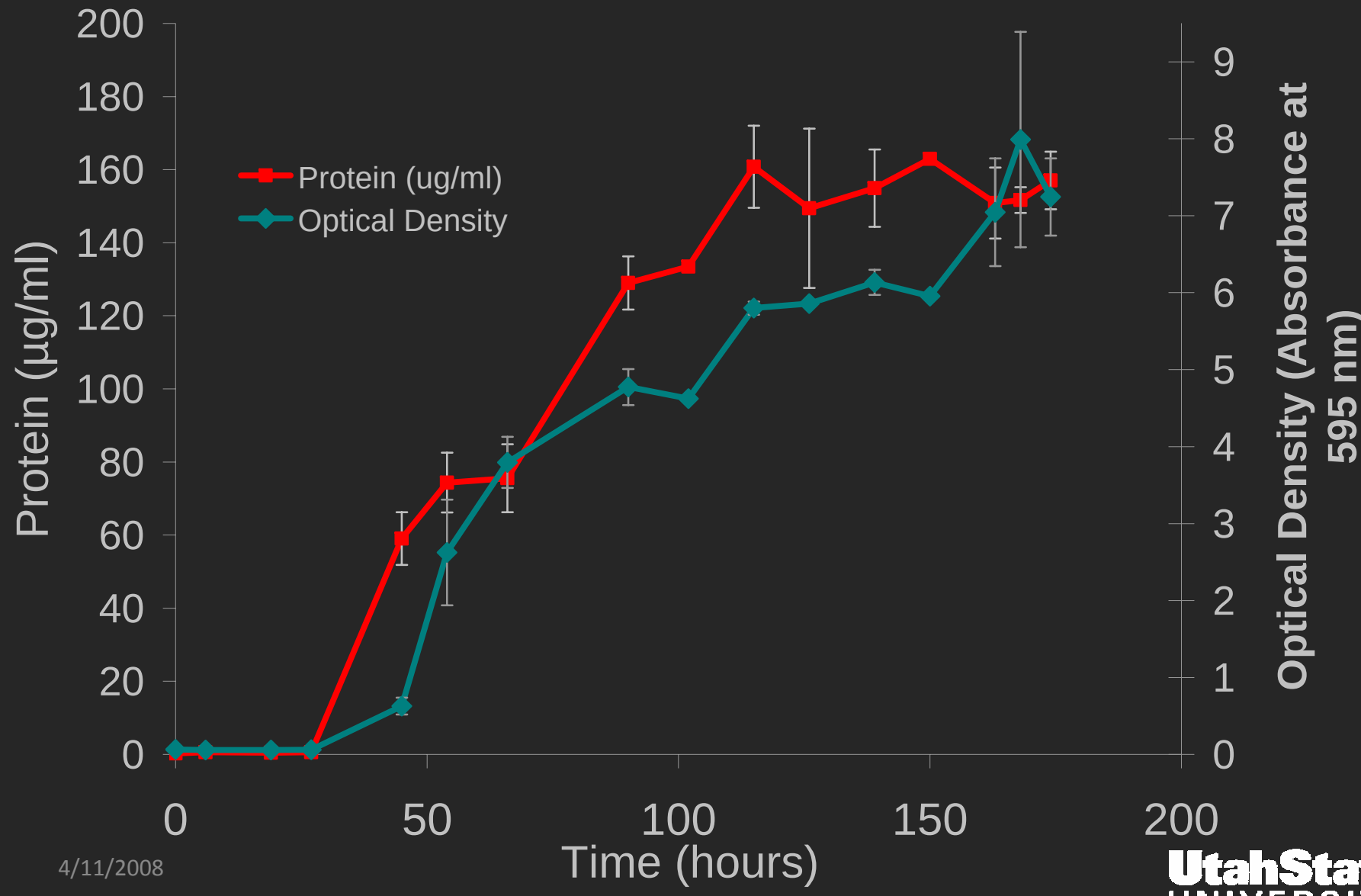
Questions?

Thank you for listening

A. vinelandii: Protein and Optical Density



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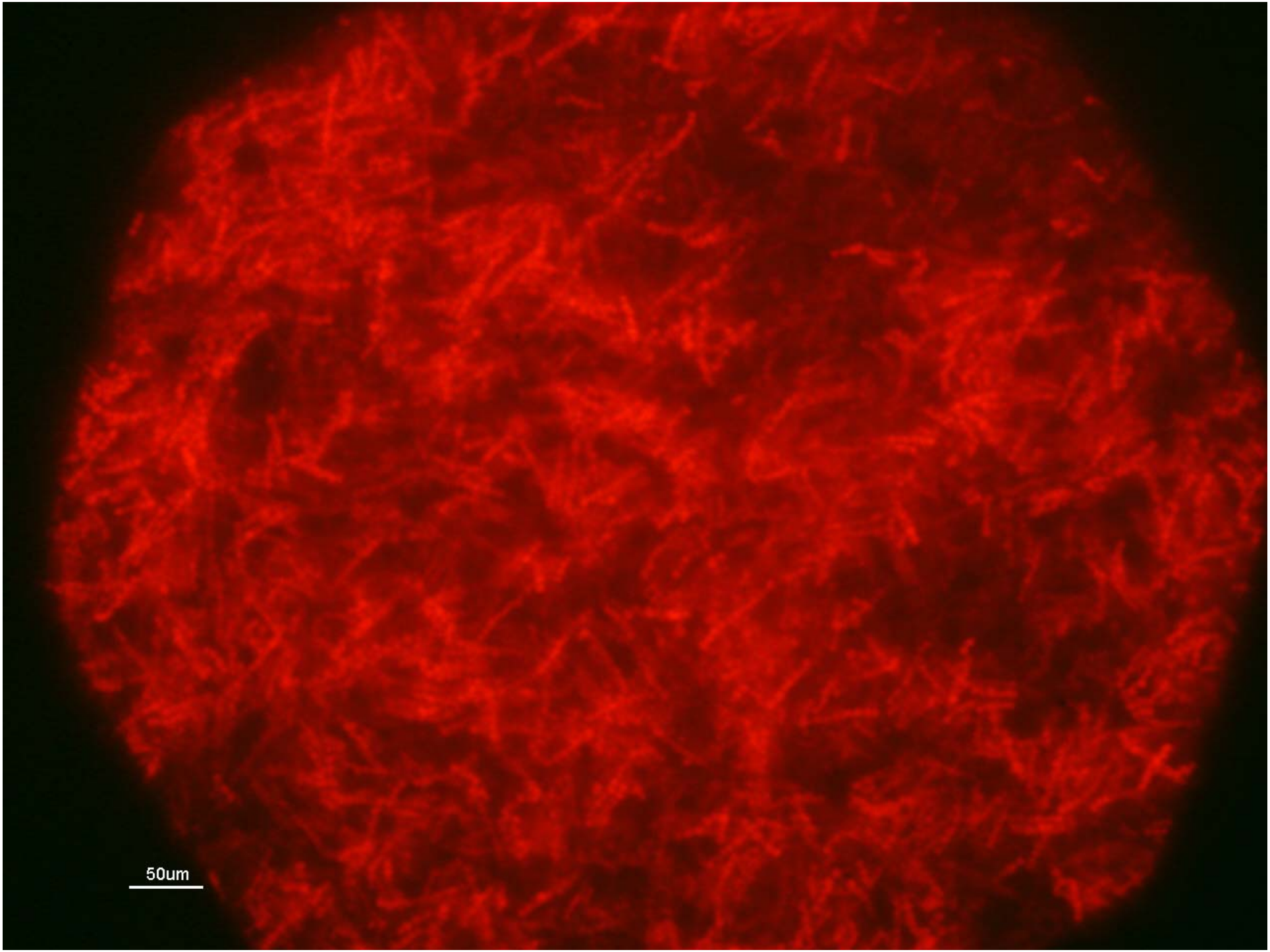
Fluorescence

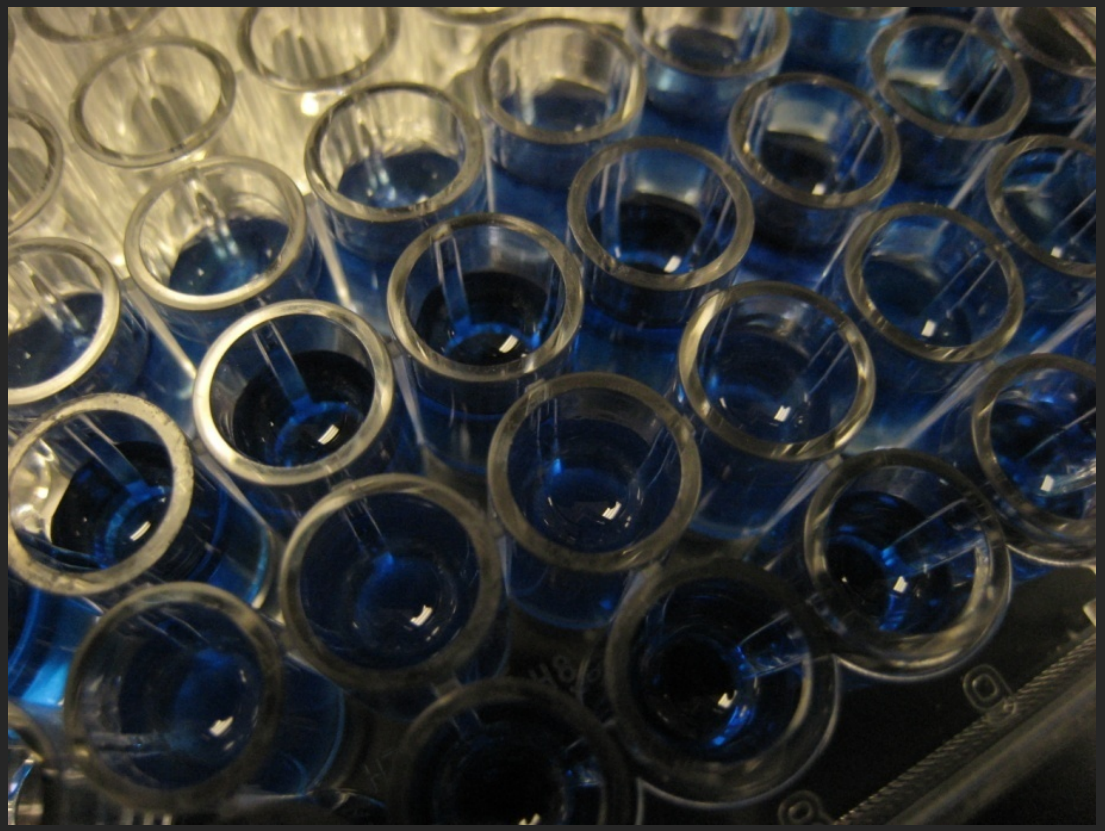
- Qualitative and intracellular detection of PHAs

- Staining with fluorescence dye
  - EXCITION: 490 nm
  - EMISSION: 580 nm

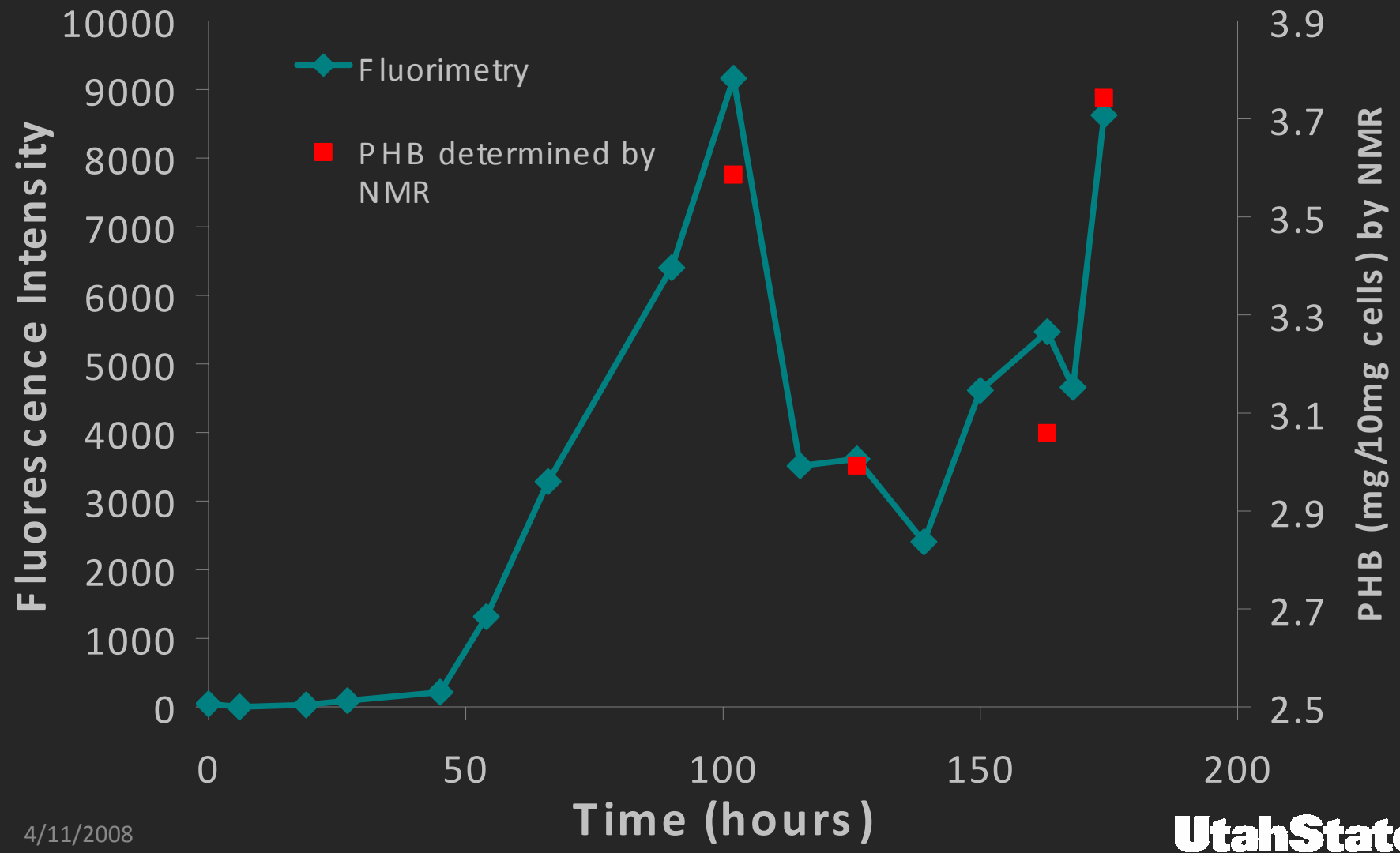

50um

Fluorimetry

- Plate reader for rapid measurements
- Inter-sample variability



NMR and Fluorimetry



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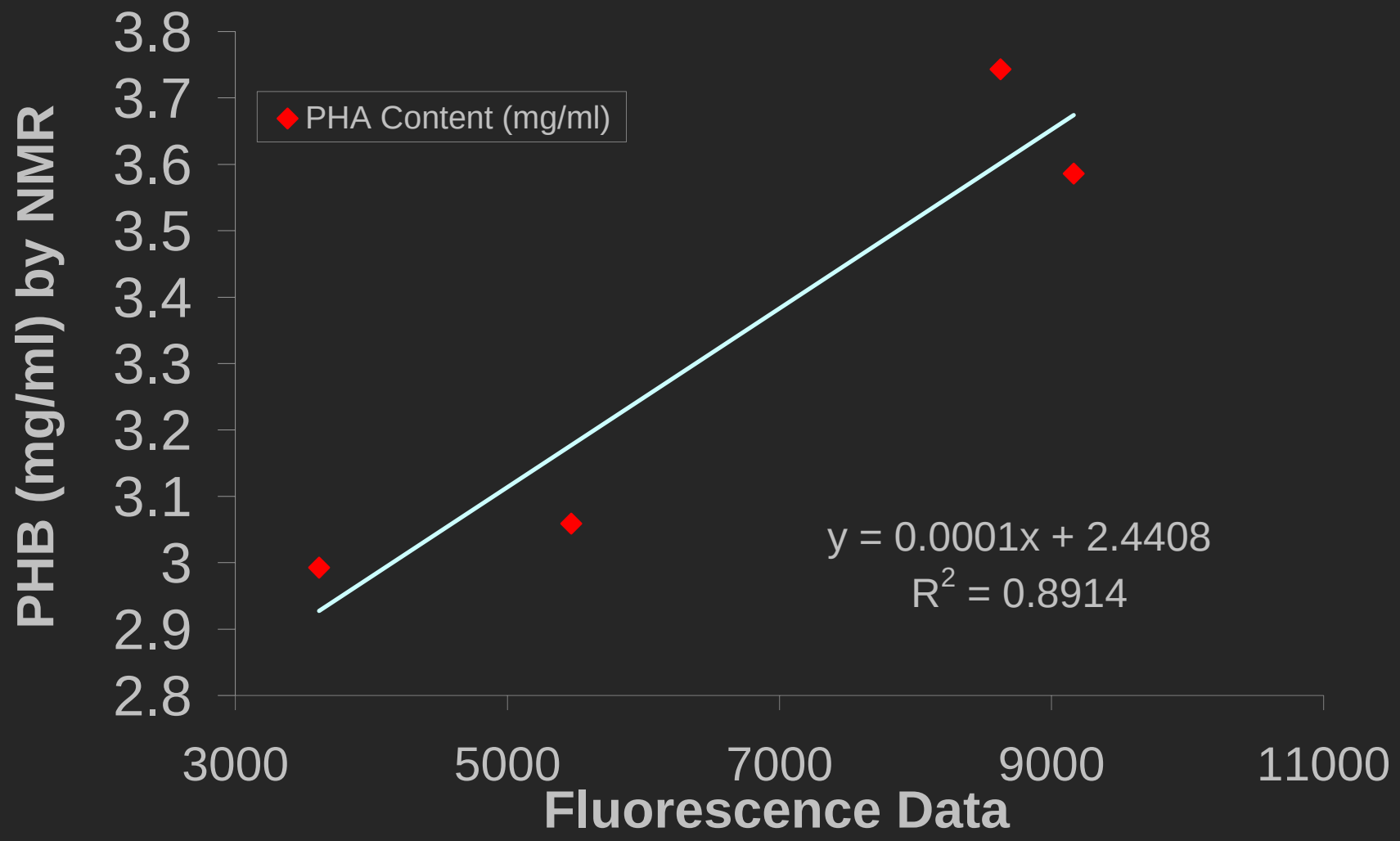
NMR vs. Fluorescence



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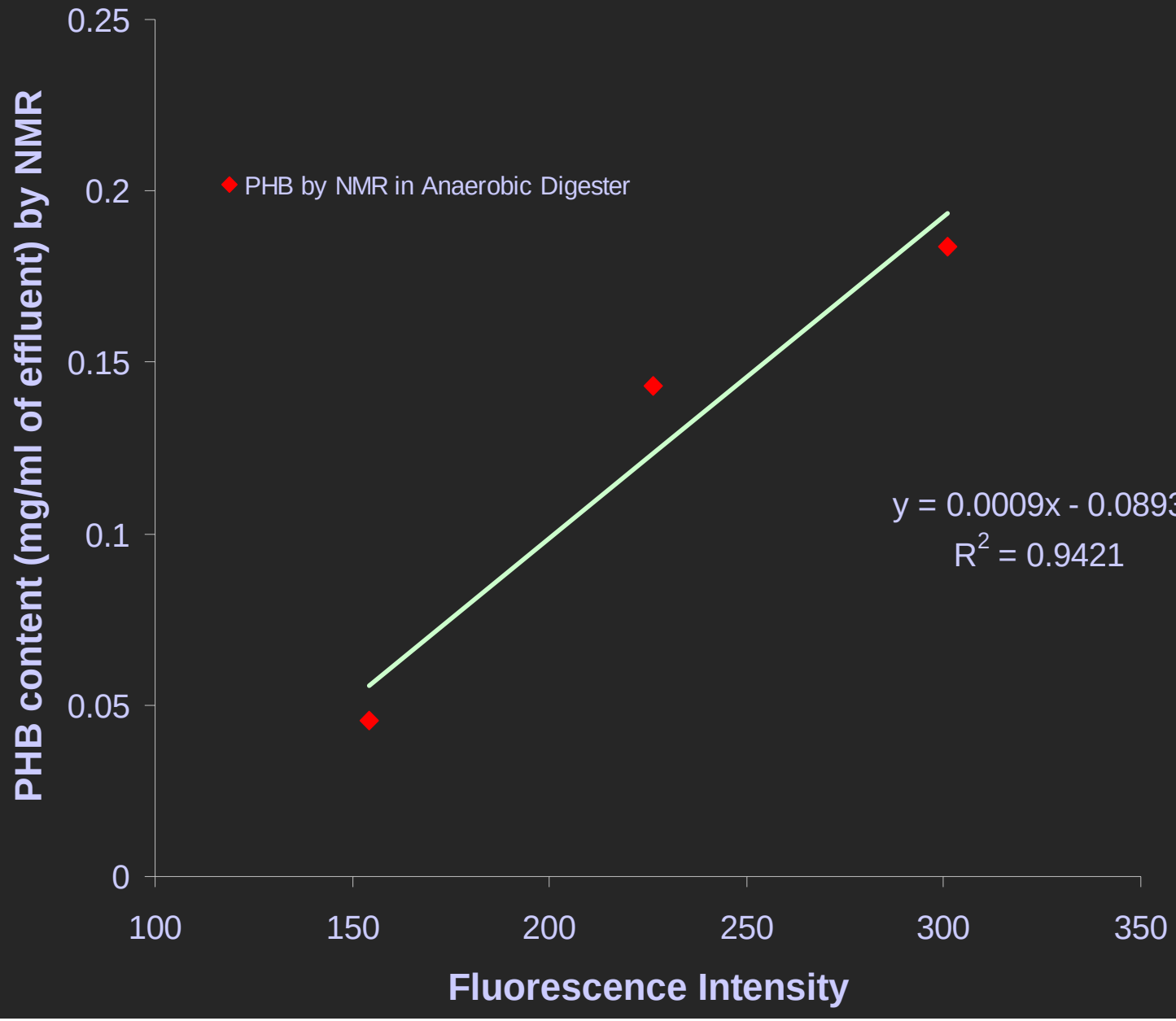
Fluorimetry with Digester Effluent



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